(a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,

(b) intentionally subjecting the operator DNA sequence to a non-naturally occurring mutagenesis, and

(c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

REMARKS

Claims 38-76 are pending in this application. By this Amendment, Claims 38, 46, 49, 52, 69, and 70 have been amended. Claim 49 stands allowed. Claims 38-48 and 50-76 have been rejected.

Claim 52 has been objected to for depending on Claim 53. Applicant submits that this is a result of a typographical error and that Claim 52 should depend upon Claim 50. Applicant has amended the claim to correct the error and requests that the rejection be withdrawn.

Claim 49 was rejected under 35 U.S.C. 101 for claiming a naturally occurring sequence. The Office Action suggested overcoming this amendment by inserting the word "isolated" prior to the "lambda O_R" terminology, and Applicant has adopted this suggestion in amended Claim 49. Accordingly, Applicant requests that the rejection be withdrawn.

Claim 38 has been rejected under 35 U.S.C. 112, first paragraph, as not enabled. In particular, the Office Action has taken issue with the phrase "non-naturally occurring." The Office Action stated that there is no support in the specification for this phrase. Applicant submits that the term is to be read in its most literal form, that is, a mutation which is not due to natural causes, or a mutation which is man-made. The specification, especially in cited pages 5-7, is replete with instances and explanations of mutagenesis that is instigated by non-natural (or man-made) means. Applicant, therefore, requests that this rejection be withdrawn.

Claim 38 has also been rejected under 35 U.S.C. 112, second paragraph, for the same claim language. Applicant submits that the term used in Claim 38 is easily understood by those of ordinary skill. Furthermore, the specification more than adequately defines and supports the above-stated definition. Accordingly, Applicant requests that this rejection be withdrawn as well.

Claims 46-48, 50-62, 66-70, and 73-76 remain rejected as obvious under 35 U.S.C. 103(a) in light of the Chen, Eliason, Pakula, Benson, the '678 patent, and the '190 patent. The Office Action advanced that the claims are directed to a method for selecting mutated O_R or O_L operator DNA sequences from lambdoid phages which have different thermostability compared to wild-type sequences, with regard to binding a repressor wherein the operator DNA sequence is subjected to mutation and selected for different thermostability from the wild type with respect to binding of a repressor. The Office Action noted that the repressor may be cI857, and the thermostability may be increased from 3-10° or 7-9° and that the claims are drawn to the mutated O_R or O_L

operator DNA sequences from lambdoid phages which may be incorporated into a vector, and to a host bacterial cell.

The Office Action also observed that Chen teaches mutated pL or oL operator DNA sequences from lambdoid phages which may be incorporated into a vector, and to a host bacterial cell, where the cI857 repressor was used to control expression by the operator sequences in a temperature dependent manner. It was conceded, though, that Chen did not teach that the mutated operator sequences had an altered binding affinity for cI857 repressor, nor that the suicide gene was from PhiX174. Additionally, it was noted that Chen failed to teach the specific temperature ranges of changes in the thermostability of the operator binding repressor, nor that the vector was a bacterial chromosomal vector or the use of multiple operator sequences.

To correct these deficiencies, the Office Action cited other references. The Eliason reference is discussed in the Office Action as disclosing a method for selecting mutated O_R or O_L operator DNA sequences from lambdoid phages which have different binding when compared to wild-type sequences, with regard to binding a repressor wherein the operator DNA sequence is subjected to mutation and selected for different binding from the wild type with respect to binding of a repressor. The reference is also stated to teach mutated O_R or O_L operator DNA sequences from lambdoid phages which may be incorporated into a vector, and to a host bacterial cell.

The Office Action also stated that the Pakula reference teaches the change in thermal stability of a mutated repressor protein with the lambda operator, and that it discusses in great detail the importance of the contact bases in the operator, and the manner in which they interact with the amino acids of the repressor protein. The Office

Action states that the discussion makes it clear that the increased thermal stability of the binding of the repressor protein is directly related to the thermodynamics of the molecular interaction between the contact bases of the operator DNA sequence and the contact amino acids of the repressor protein. It was also advanced by the Office Action that the Pakula reference teaches that one of skill in the art would be able to select mutated sequences in the repressor protein which would have greater binding affinity for the operator sequences, and, therefore, higher thermostability.

The Office Action also alleged that the Benson reference taught the relative affinity of the lambda repressor protein for the lambda operator sequence, where the operator sequence has been mutated. Benson is stated to show that the operator sequence was mutated to produce a mutated operator sequence which has greater affinity for the lambda repressor protein than the wild type operator sequence.

The Office Action cited two United States Patents, stating that the '678 patent taught the use of two or more operator sequence which have different affinities for the cI857 repressor in a single construct to produce different affinities for the cI857 repressor, and that the '190 patent teaches the mutation of OL operator sequences to increase the binding affinity of the cI857 repressor protein.

In light of these references, the Office Action maintained the allegation that the present invention would have been obvious to one of ordinary skill in the art.

However, Applicant notes that many of the arguments raised in the prior Response were not addressed in the pending Office Action. Some of these arguments include the discussion of the Pakula reference's definition of "thermal stability" (see page 3 of Response dated May 10, 2001), the discussion of the "Mutant Screen"

section of Pakula (see page 3 of same), the teaching away of the Pakula reference (see page 4 of same), and the applicability of the Chen reference in light of the Claim amendments (see page 6 of same). Applicant requests that either the rejection be withdrawn in light of the remaining issues or that an explanation be given as to why they were insufficient to overcome the rejection.

Applicant also submits that this rejection, as well as the previous rejections, is not well taken in light of the attached executed Declaration by Dr. Werner Lubitz, a named inventor of the present application, and advances that the explanations made within fully overcome the rejections made on this point. Applicant particularly notes paragraph 2 which explains the differences between the present invention that those used in the art. Further, the testing data contained within the Declaration clearly shows the advantages of the present invention over that already known in the art. Accordingly, Applicant would submit that the Declaration overcomes the above rejection.

Finally, Applicant would advance that this rejection is based upon the impermissible use of hindsight. Applicant submits that the combination of references cited in this rejection would only be combined by one of ordinary skill if that person already had prior knowledge of the invention. Furthermore, Applicant reasserts that Pakula describes mutations in a Cro repressor protein whereas the present invention is directed towards mutations in the DNA sequence of the cI operator sequence. Thus, Applicant submits that not only is Pakula an inappropriate reference for this application, but that given its vast differences from the present application, any reliance upon it by one of ordinary skill of the art would be based upon hindsight (as it does not relate to the present invention).

Claims 38-48, 50-62, 66-70, and 73-76 were rejected under 35 U.S.C. 103(a) as obvious in light of Chen, Eliason, Pakula, Benson, the '678 patent, the '190 patent, and United States Patent No. 5,811,093. The Office Action used the description of the previous references and stated that the '093 patent teaches a mutator bacterial strain used for the well-known mutation of a desired sequence of phage DNA.

The Office Action alleged that it would have been obvious to one of ordinary skill in the art to use a mutator strain of bacteria like the one presented in the '093 patent to produce mutations in a selected DNA sequence such as the claimed lambda operator sequence. The Office Action also responded to arguments from the last two Responses directed towards this ground of rejection. The Office Action noted that Eliason taught that the mutated operator sequences have a higher affinity for the cI857 repressor and that Pakula taught the thermodynamics of the interaction of a repressor with mutated operator sequences. The Office Action stated that Pakula makes it very clear that an increase in affinity of the repressor for the operator sequences will also increase the thermostability of the repressor as it binds to the mutated operator sequences.

The Office Action also responded to the arguments that the Pakula reference had been misinterpreted. The Office Action replied in the pending Office Action that the Pakula teachings are directed towards the thermostability of the repressor protein, and that it teaches important general knowledge of the basic thermodynamics of molecular binding, and its general results on the thermostability of a DNA/repressor binding complex. The Office Action maintained that repressor protein binding to the operator DNA sequences must follow the laws of thermodynamics set forth in the Pakula reference. The Office Action advanced that Pakula teaches that the binding affinity of a

repressor protein to lambda operator sequences is affected by changes in the lambda operator DNA sequence, and that the thermostability of the operator/DNA complex is altered by changes in the binding affinity. The Office Action advanced that this is well known information regarding the thermodynamics of protein/DNA binding and that stronger binding affinity results in higher thermostability of the complex and that weaker binding affinity results in a lower thermostability.

The Office Action also stated that the arguments advancing that Benson did not teach mutated operator sequences having increased thermostability were not convincing. The Office Action alleged that Benson taught that repressor bound to mutated operator sequences with different affinities with respect to the changes in the DNA sequence, and that the different binding affinities of the mutated operator sequences must result in different thermal stabilities of the repressor/operator complex. The Office Action advanced that the fact that Benson does not mention thermostability is irrelevant to the basic physical principles which underlie the binding affinities, and that requiring Benson to specifically mention this fact is not necessary to make the point because the physical principles must apply in all cases.

While the Office Action may be correct on the issue of Benson not having to specifically mention the term "thermostability," Applicant submits that the Office Action has mischaracterized Applicant's comments on this source. Applicant reiterates that the Benson reference fails to disclose a reliable means of producing a mutant operator sequence such as the one claimed, much less the claimed mutated operator sequence and resubmits its request that the rejection be withdrawn.

The Office Action also rejected arguments that the '190 patent taught "tighter regulation." The Office Action noted that pHDM159 contains a change that increases the binding affinity for the cI857 repressor, and that changes in the binding affinity must also affect the thermostability of the complex. Applicant notes that the Office Action has, again, relied upon the Pakula teachings, even though these teachings fail to teach the theories the Office Action purports to rely upon.

The Office Action also refuted arguments that stated that amending Claim 38 to recite "non-naturally occurring mutagenesis" distinguished the claim from Chen. The Office Action replied that the teachings set forth in the '093 patent regarding the use of a mutator bacterial strain provided sufficient motivation to combine the references to mutate the mutated sequences of Chen to provide the mutated operator sequences of the instant claimed invention.

Applicant fails to see why one with skill in the art would be motivated to combine the '093 patent with Chen. '093 is directed to suppression of an immune system and not towards operator mutations. While some of the theories discussed in the '093 patent may have some bearing on the present invention, Applicant does not discern any true motivating statement in either Chen or the '093 reference to combine the two.

Applicant also submits that this rejection is not well taken in light of the attached executed Declaration by Dr. Werner Lubitz, a named inventor of the present application, and advances that the explanations made within fully overcome the rejections made on this point. Applicant particularly notes paragraph 2 which explains the differences between the present invention that those used in the art. Further, the testing data contained within the Declaration clearly shows the advantages of the

present invention over that already known in the art. Accordingly, Applicant would submit that the Declaration overcomes the above rejection.

Applicant submits that this rejection is also based upon the impermissible use of hindsight. Applicant submits that the combination of references cited in this rejection would only be combined by one of ordinary skill if that person already had prior knowledge of the invention. Additionally, Applicant resubmits that Pakula describes mutations in a Cro repressor protein whereas the present invention is directed towards mutations in the DNA sequence of the cI operator sequence. Thus, Applicant submits that not only is Pakula an inappropriate reference for this application, but that given its vast differences from the present application, any reliance upon it by one of ordinary skill of the art would be based upon hindsight (as it does not relate to the present invention).

The Office Action also rejected Claims 38-48 and 50-76 as obvious under 35 U.S.C. 103(a) in light of the Chen, Eliason, Pakula, Benson, and Szostak references, as well as the '678, and '190 patents. The Office Action noted that the rejected claims were directed towards the same subject matter as described above and a vaccine composition comprising the bacterial cell, and bacterial cell ghosts produced by transfecting bacterial cells with the above claimed compositions and methods.

The Office Action conceded that the above references, without the Szostak reference, did not teach the vaccine composition comprising the bacterial cell and bacterial cell ghosts produced by transfecting bacterial cells with the above claimed compositions and methods. However, it was noted that Szostak taught vaccines made by transfecting bacterial cells with the above claimed compositions and methods. The

Office Action alleged that it would have been obvious to one of ordinary skill to combine the composition comprising the transfecting of bacterial cells with the above claimed compositions and methods with the vaccine composition of Szostak.

Applicant again submits that this rejection, as well as the previous rejection, is not well taken in light of the attached executed Declaration by Dr. Werner Lubitz, a named inventor of the present application, and advances that the explanations made within fully overcome the rejections made on this point. Applicant particularly notes paragraph 2 which explains the differences between the present invention that those used in the art. Further, the testing data contained within the Declaration clearly shows the advantages of the present invention over that already known in the art. Accordingly, Applicant would submit that the Declaration overcomes the above rejection.

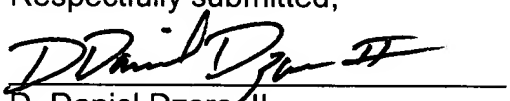
Finally, Applicant would advance that this rejection is based upon the impermissible use of hindsight. Applicant submits that the combination of references cited in this rejection would only be combined by one of ordinary skill if that person already had prior knowledge of the invention. Furthermore, Applicant reasserts that Pakula describes mutations in a Cro repressor protein whereas the present invention is directed towards mutations in the DNA sequence of the cI operator sequence. Thus, Applicant submits that not only is Pakula an inappropriate reference for this application, but that given its vast differences from the present application, any reliance upon it by one of ordinary skill of the art would be based upon hindsight (as it does not relate to the present invention).

Therefore, Applicant respectfully submits that the application is in condition for allowance and requests that all rejections be withdrawn.

In the event this paper is not timely filed, Applicant hereby petition for an appropriate extension of time. The fee for this extension may be charged to Applicant's Deposit Account No. 01-2300.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300, referring to client-matter number 100564-09005.

Respectfully submitted,



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Attachments: Marked Up Copy of Claims
Petition for Extension of Time
Executed Declaration

MARKED UP COPY OF CLAIMS

38. (twice amended) A method for selecting OR or OL operator DNA sequences from lambdoid phages wherein said sequences have a different thermostability compared to a wild-type sequence with regard to binding a repressor, wherein said different thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, comprising

(a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,

(b) intentionally subjecting the operator DNA sequence to a non-naturally occurring mutagenesis, and

(c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

46. (twice amended) An OR or OL operator sequence from lambdoid phages which have an increased thermostability compared to a wild-type sequence with regard to binding of a temperature-sensitive cI repressor, wherein said increased thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said sequences are obtained by a method comprising

(a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,

(b) intentionally subjecting the operator DNA sequence to a non-naturally occurring mutagenesis, and

(c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

49. (amended) An isolated lambda OR operator sequence comprising the sequence shown in SEQ ID NO. 2.

52. (amended) The nucleic acid according to claim [53] 50, wherein the expression control sequence contains a lambda PL or PR promoter.

69. (amended) The bacterial cell according to claim 67, wherein said first bacterial expression control sequence is an operator sequence from a lambdoid phage wherein said sequence has a different thermostability compared to a wild-type sequence with regard to binding of a repressor wherein said different thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said operator sequence is obtained by a method comprising

(a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,

(b) intentionally subjecting the operator DNA sequence to a non-naturally occurring mutagenesis, and

(c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

70. (amended) The bacterial cell according to claim 67, further comprising (c) a third bacterial expression control sequence which contains a operator sequence in operative linkage with a suicide gene, wherein said operator sequence is from a lambdoid phage and wherein said operator sequence has a different thermostability compared to a wild-type sequence with regard to binding of a repressor, wherein said different thermostability results in repression of expression of a gene which is

operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said operator sequence is obtained by a method comprising

(a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,

(b) intentionally subjecting the operator DNA sequence to a non-naturally occurring mutagenesis, and

(c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

#20

LUBITZ et al.

Art Unit: 1636

Serial Number: 09/147,693

Examiner: W. Sandals

Filed: February 17, 1999

Atty. Docket No. 100564-09005

For: NEW SYSTEMS FOR REGULATION OF GENE EXPRESSION

DECLARATION

Commissioner for Patents
Washington, D.C. 20231

Date: January ___, 2002

Sir:

I, Professor Werner Lubitz, a citizen of Germany, hereby declare and state:

1. I am a co-inventor of the above-identified application and am familiar with the rejections made by the examiner, as well as the references relied upon for the rejections.

2. A basic principle of the above-referenced application is as follows. The interaction between the wild-type operator sequence and the temperature sensitive cI mutant is based on the fact that when exposed to a temperature of 28°C to 30°C, the repressor mutant stably binds to the operator. When the temperature is raised, for example to 36°C or 42°C, the binding between the repressor and the operator is

destabilized. Due to this destabilization, the blocking of gene expression is offset by the promotor located next to the operator, and gene expression is induced.

The wild-type operator sequence/temperature-sensitive cI repressor has been employed for many years to regulate gene expression, whereby, first, the cell is cultivated under non-inducing conditions (i.e., at 28° or 30°C), and then the gene expression is induced by raising the temperature to 36°C or 42°C. This system, however, has a decisive drawback. That is, the range of stability for the binding of the cI repressor to the operator is too small to permit the cultivation of microorganisms under optimal conditions because these conditions would involve a temperature, for instance, within the range of 33-37°C. The present invention, however, has solved this problem by modifying the operator sequence so that the stability of the binding between the repressor and operator is optimized in such a manner that an improved system for the temperature-inducible gene expression is provided. Prior to the present invention, it was not known that operator mutants existed which fulfill the prerequisites for such a gene expression system together with a temperature-sensitive cI repressor.

3. I carried out tests comparing an operator mutant according to the invention (WJ) with the operator wild-type and a prior art mutant (C10). As described in the present application, the mutant of the invention shows 6 to 7°C higher thermostability with regard to binding a temperature-sensitive C1 repressor compared to the wild-type mutant. The C10 mutant shows a further increased thermostability of the

binding of the repressor mutant, i.e., the operator C10 is substantially repressed up to 42°C.

The consequence of these tests is as follows:

In my opinion, none of the cited prior art documents renders obvious the claimed process for the systematic identification of λ-operator mutants having increased thermostability compared to the wild-type. In particular, the prior art does not include any hint as to the preparation of a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one O_R or O_L operator DNA sequence from a lambdoid phage in a promotor in connection with mutagenesis step (b) and analysis step (c).

The product claims are directed to operator sequences having 3 to 10°C higher thermostability than the wild-type. Prior art mutant C10 does not fulfill this feature because it does not allow inducible gene expression, even if the temperature is increased by 12°C as compared to the temperature at which repressor binding to the wild-type is still stable (30°C).

The mutants according to the invention, however, surprisingly allow cultivation under repressible conditions in a temperature range especially favorable for the cultivation of bacterial cells. If said temperature limit is exceeded, e.g., temperature increase to 42°C, gene expression is strongly induced. In contrast to this, prior art mutant C10 does not allow inducible gene expression at 42°C, as can be seen from the enclosed Figures, i.e., the mutant is unsuitable for a process of inducible gene

expression. In the case of the wild-type sequence binding of the temperature-sensitive repressor to the operator, it is stable only up to 30°C. Therefore, fermentation under repressible conditions is possible only at a temperature at which the bacterial cells do not grow well.

The experimentation data I collected are explained in detail in the enclosed Figures. Fig. 1 shows the various plasmid constructs. Temperature-dependent expression of the various operator sequences is investigated.

Fig. 2 shows that the wild-type is induced most at a temperature shift from 28°C to 42°C. The mutant of the invention is strongly induced as well. Prior art mutant C10, however, does not show any significant induction. Therefore, it can be gathered from the Figure that all three of the constructs do not exhibit significant gene expression when cultivated at 28°C. However, when the temperature is shifted to 42°C, the expression of the wild-type construct is induced, as well as the expression of the current invention. The operator mutant of the prior art is hardly induced and, therefore, is not at all suitable for producing an inducible gene expression system.

Fig. 3 shows a temperature shift of the three constructs from 28°C to 36°C. As expected, the wild-type construct is strongly induced because the wild-type operator has no temperature stability above 30°C with regard to binding the temperature-sensitive repressor. The construct of the invention and the prior art construct, however, are not significantly induced upon temperature shift to 36°C. This proves that the thermostability of the present invention is significantly higher than that of the wild-type

sequence. As seen in Figure 2, by raising the temperature up to 42°C, the binding can be destabilized so that gene expression is induced. The prior art sequence, however, does not exhibit any such inducement. The prior art sequence thermostability is so strong that an increase in gene expression by means of temperature shift is not possible.

Thus, a comparison of Figs. 2 and 3 shows that the construct of the invention has about a 5 to 6°C higher thermostability than the wild-type operator with regard to binding a temperature-sensitive repressor. The construct of the invention can therefore be used in fermentation processes at a temperature of optimal microorganism cultivation, under repressing conditions. In the case of a temperature shift to 42°C (more than 10°C above the stability limit of the wild-type sequence) there is strong induction. In contrast to this, the prior art construct does not show any significant induction, even if the temperature is shifted to 42°C. Said prior art construct, thus, is not suited for regulatable gene expression under common culture conditions.

4. From the foregoing, it can be concluded that the construct of the present invention exhibits a thermostability higher by 5 to 10°C as compared to the wild type construct. However, unlike the sequence of the prior art which also has a higher thermostability, the present invention remains suitable for induced gene expression. The presence of such operator mutants showing these features could not be derived from the prior art.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

W. Lubitz

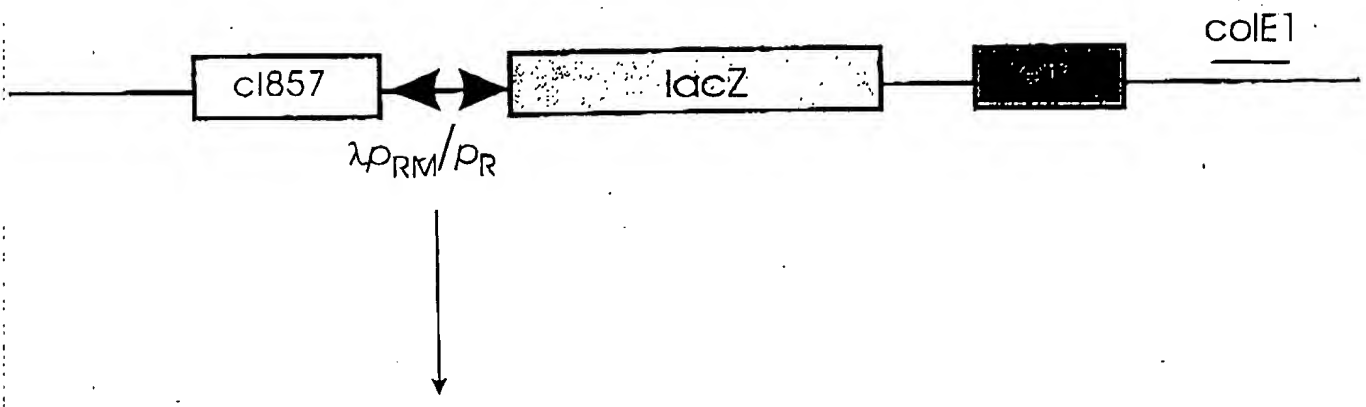
Professor Werner Lubitz

11 February 2002

Date

Enclosures: Figs. 1-3

Fig.1



pAW-lac : wt $\lambda p_{RM}/p_R$ (wild-type)

pAWJ-lac : mut $\lambda p_{RM}/p_R$ from pAWJ (invention)

pAWC10-lac : mut $\lambda p_{RM}/p_R$ from pAWC10 (prior art)

Fig.2

time [min]	AW-lac	AWJ-lac	AWC10-lac	AW -	AW +	
-30	121	95	119	118	125	28°C
0	99	78	99	99	100	28°C/SHIFT
30	5257	2854	689	5253	5262	42°C
60	38131	24157	1794	37684	38578	42°C
90	54652	34703	3096	53693	55611	42°C

time [min]	AW-lac	AWJ-lac	AWC10-lac	AWJ -	AWJ +
-30	121	95	119	95	96
0	99	78	99	78	79
30	5257	2854	689	2815	2893
60	38131	24157	1794	23675	24640
90	54652	34703	3096	33184	36222

time [min]	AW-lac	AWJ-lac	AWC10-lac	AWC10 -	AWC10 +
-30	121	95	119	93	145
0	99	78	99	82	117
30	5257	2854	689	606	772
60	38131	24157	1794	1605	1983
90	54652	34703	3096	3084	3109

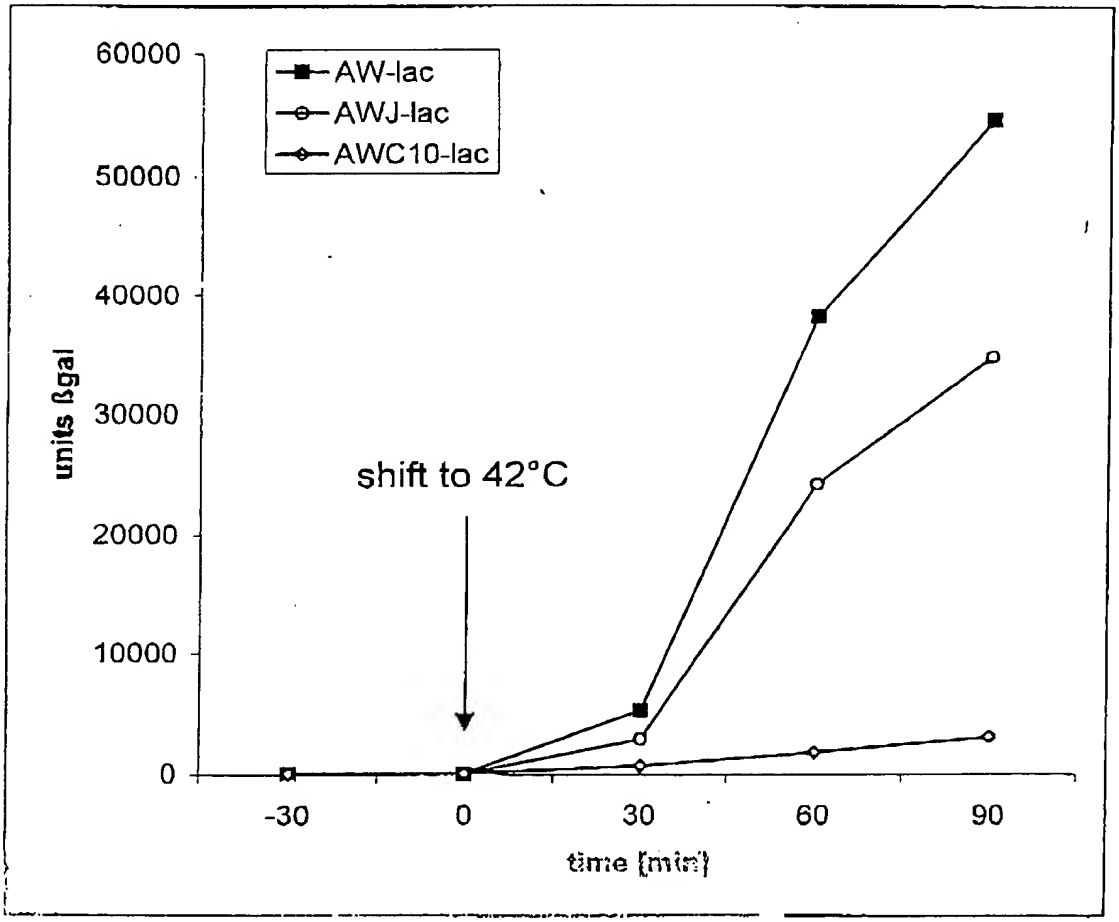


Fig.3

time [min]	AW-lac	AWJ-lac	AWC10-lac	AW -	AW +
-60	161	170	157	137	186
0	118	125	123	106	130
30	1577	365	185	1274	1880
60	5046	702	213	4386	5706
105	8729	1141	367	8725	8733

time [min]	AW-lac	AWJ-lac	AWC10-lac	AWJ -	AWJ +
-60	161	170	157	165	170
0	118	125	123	120	130
30	1880	365	185	335	395
60	5706	702	213	579	826
105	8733	1141	367	986	1296

time [min]	AW-lac	AWJ-lac	AWC10-lac	AWC10 -	AWC10 +
-60	161	170	157	155	159
0	118	125	123	123	123
30	1880	365	185	184	187
60	5706	702	213	208	219
105	8733	1141	367	360	374